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Variable indoleamine 2,3-dioxygenase expression in acral/mucosal melanoma and its possible link to immunotherapy

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Abstract

Immune checkpoint inhibitors have improved the prognosis of advanced melanoma. Although anti–programmed death ligand-1 (PD-L1) is a well-studied biomarker for response to anti–programmed death-1 PD-1 therapy in melanoma, its clinical relevance remains unclear. It has been established that the high expression of indoleamine 2,3-dioxygenase (IDO) is correlated to a response to anti–CTLA-4 treatment in melanoma. However, it is still unknown whether the IDO expression is associated with response to anti–PD-1 therapy in advanced melanoma. In addition, acral and mucosal melanomas, which comprise a great proportion of all melanomas in Asians, are genetically different subtypes from cutaneous melanomas; however, they have not been independently analyzed due to their low frequency in Western countries. To evaluate the association of IDO and PD-L1 expression with response to anti–PD-1 antibody in acral and mucosal melanoma patients, we analyzed 32 Japanese patients with acral and mucosal melanomas treated with anti–PD-1 antibody from the perspective of IDO and PD-L1 expression levels by immunohistochemistry (IHC). Multivariate Cox

regression models showed that the low expression of IDO in tumors was associated with poor progression-free survival (HR = 0.33, 95% CI = 0.13-0.81, $P = 0.016$), whereas PD-L1 expression on tumors was not associated with progression-free survival. Significantly lower expression of IDO in tumors was found in non–responders compared to responders. Assessment of the IDO expression could be useful for the identification of suitable candidates for anti–PD-1 therapy among acral and mucosal melanomas patients. Further validation study is needed to estimate the clinical utility of our findings.

KEYWORDS
3-dioxygenase, checkpoint inhibitor, IDO, indoleamine 2, melanoma, PD-1

1 | INTRODUCTION

Immune checkpoint inhibitors have revolutionized the treatment of advanced melanomas. Anti–cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) and anti–programmed death-1 (PD-1) therapy provided durable responses in approximately 10% and 33%-40% of advanced melanoma patients, respectively,[1-3] indicating that immune checkpoint inhibitors are effective for only a certain subset of patients. In addition, immune checkpoint inhibitors frequently exhibit immune-related cytotoxicities.[1-3] Therefore, predictive biomarkers for responses to immune checkpoint inhibitors are urgently needed to select suitable candidates. Although programmed death ligand-1 (PD-L1) is a well-studied biomarker for response to anti–PD-1 therapy in melanoma, there are contradicting reports on its clinical relevance.[4,5] Several reports show that other possible biomarkers could predict the response to treatment with anti–PD-1 antibody in melanomas, such as tumor-infiltrating CD8+ T cells, *BRCA2* mutation, HLA-A allele, monocytes in blood, and blood neutrophil-to-lymphocyte ratio combined with serum lactate dehydrogenase (LDH) level.[6-10] However, the relevance of these biomarkers in clinical practice and their routine application remain unclear.

Indoleamine 2,3-dioxygenase (IDO) is a critical step in the kynurenine pathway that metabolizes tryptophan.[11,12] IDO shows immune-suppressive activities by negatively modulating effector T cell function and enhancing the regulatory T cell activities through the tryptophan metabolites.[11,12] IDO is expressed in tumor cells, dendritic cells, macrophages and endothelial cells in the tumor microenvironment.[12] IDO is expressed in both tumor cells and immune cells in melanomas.[13] A positive correlation between the high expression of IDO and clinical response to anti–CTLA-4 therapy in melanoma has been reported.[14] However, the association of IDO expression with response to anti–PD-1 therapy in melanoma remains unclear.

Acral and mucosal melanomas have not been analyzed independently from other types of melanomas in most clinical trials due to their low frequency in Western countries; however, they comprise a great proportion of all melanomas diagnosed in Asians.[2,3,15-19] Hayward et al[20] show that acral and mucosal melanomas differ starkly from cutaneous melanomas in terms of mutational burden, structural variant, mutational signature and driver mutations. Therefore, focusing on acral and mucosal melanomas may provide insights specific to these subtypes.

In this study, we analyzed Japanese patients with acral and mucosal melanomas treated with anti–PD-1 antibody. Immunohistochemistry (IHC) was performed to assess the association of the IDO and PD-L1 expression with response to anti–PD-1 therapy.

2 | MATERIALS AND METHODS

2.1 | Patients and samples

Eligible patients were those with unresectable acral or mucosal melanomas who initiated anti–PD-1 therapy between 2015 and 2017 at the Kyoto University Hospital and 8 participating hospitals. Other eligibility criteria included an Eastern Cooperative Oncology Group (ECOG) performance status (PS) score of 0 or 1, and the availability of formalin-fixed, paraffin-embedded tumor specimens within 2 months before the first treatment of anti–PD-1 antibody nivolumab. Patients received anti–PD-1 therapy at either 3 mg/kg dosing every 2 weeks or at 2 mg/kg dosing every 3 weeks. Tumors were assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1.[21] Responders were defined as patients who had a complete response (CR) or partial response (PR) as their best overall response. The objective response rate (ORR) was defined as the proportion of patients who achieved a CR or PR as their best overall response. This study was approved by the ethics committee of the Kyoto University Graduate School of Medicine and participating institutions. Written informed consent was obtained from all patients.

2.2 | Immunohistochemical analysis

Immunohistochemistry was performed using formalin-fixed, paraffin-embedded tumor specimens with BOND RX Fully Automated

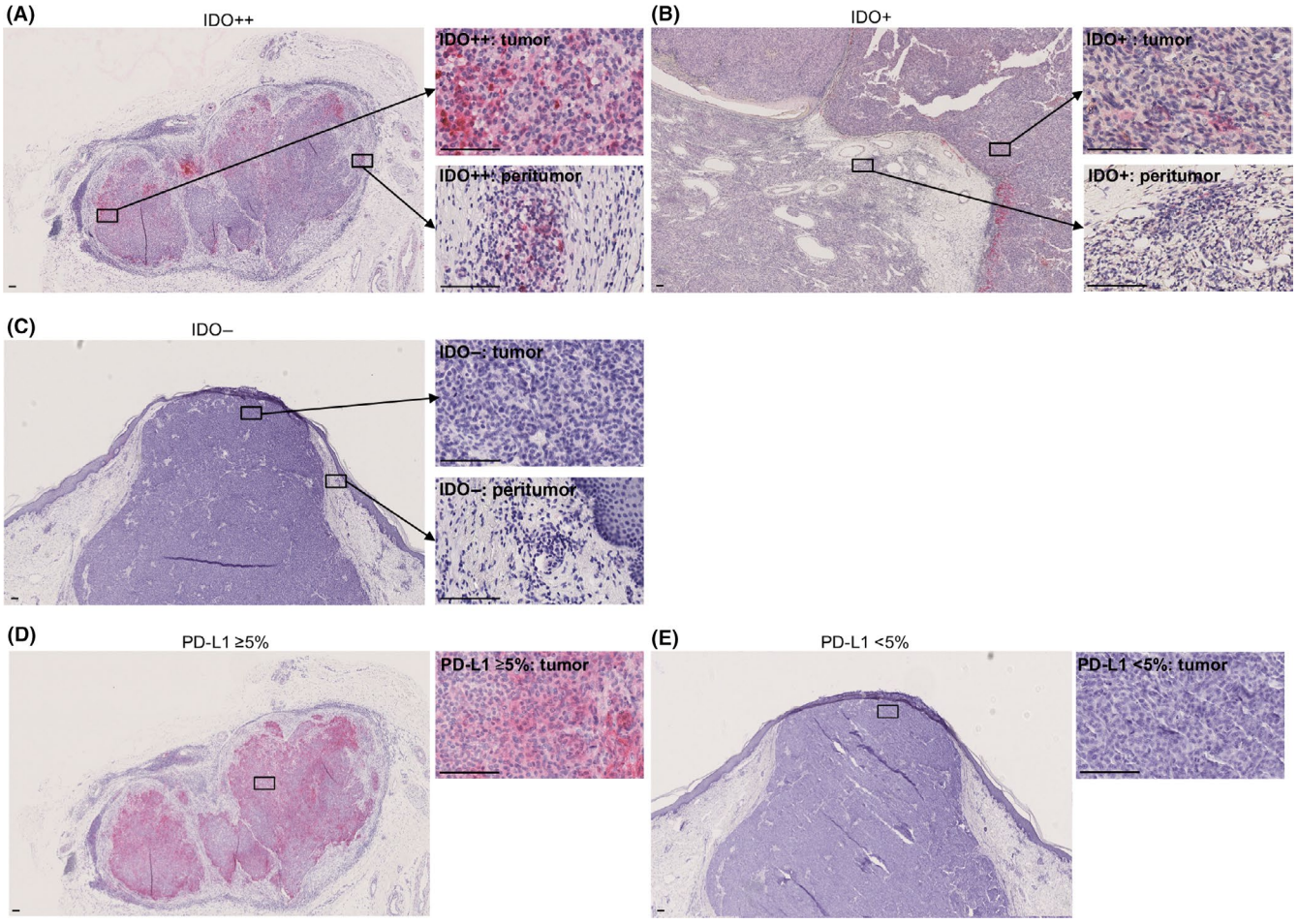

FIGURE 1 Indoleamine 2,3-dioxygenase (IDO) and programmed death ligand-1 (PD-L1) expression by immunohistochemistry (IHC). Representative IHC images of IDO++, IDO+ and IDO− in tumors and in peritumoral mononuclear inflammatory cells are shown (A-C). Representative IHC staining of PD-L1(≥5%) and PD-L1(<5%) on tumors are shown (D, E)

Research Stainer (Leica). Antigen retrieval was performed using BOND Epitope Retrieval Solution 2 (Leica). In the IDO analysis, slides were incubated with the primary antibody against IDO (Clone 10.1; Merck Millipore) at a 1:250 dilution for 15 minutes. Mouse IgG1, kappa (Clone MOPC-21; BioLegend) was used as an isotype control. Signals were generated by BOND Polymer Refine Red Detection (Leica). In PD-L1 analysis, slides were incubated with the primary antibody against PD-L1 (Clone SP142; Spring Bioscience) at a 1:100 dilution for 60 minutes. Rabbit polyclonal IgG (ab27478; Abcam) was used as an isotype control. Next, the ImmPRESS-AP Anti–Rabbit IgG Polymer Detection Kit (Vector Laboratories) was used as a second antibody. Signals were generated by ImmPACT Vector Red Alkaline Phosphatase Substrate (Vector Laboratories). The sections were counterstained with hematoxylin.

2.3 | Scoring of the indoleamine 2,3-dioxygenase and anti–programmed death ligand-1 expression in melanoma

The images were captured with an automated slide scanner, Nanozoomer (Hamamatsu Photonics). In the previous reports, IDO− was defined as IDO negative, IDO+ as IDO staining in <25% of tumor or antigen-presenting cells, IDO++ as IDO staining in 25%-50% and IDO+++ as IDO staining in >50%.[13,22] Because there was no case matching IDO++ in our study, we modified the criteria as follows: IDO− as IDO negative, IDO+ as IDO staining in <25% of tumor or peritumoral mononuclear inflammatory cells and IDO++ as ≥25% (Figure 1A-C). Melanoma patients were dichotomized into the high IDO expression group as IDO++ and the low IDO expression group as IDO− and IDO+. Endothelial IDO expression in the peritumoral stroma was dichotomized as “present” or “absent” following the criteria described previously.[23] PD-L1 positivity was defined as a membranous PD-L1 staining in at least 5% of tumors (Figure 1D,E).[2,24]

2.4 | Statistical analysis

Survival curves were generated using the Kaplan-Meier method. Progression-free survival (PFS) was defined as the time from the first treatment of anti–PD-1 antibody to documented tumor progression or death. Patients were censored if they showed no sign of the disease progression on the last evaluation or if the patients were lost to follow

TABLE 1 Patient characteristics and association with IDO expression in tumors

Variables	Total (n = 32)	IDO expression in tumor			*P*-value
		IDO− (n = 15)	IDO+ (n = 6)	IDO++ (n = 11)	
Age					
≤65 y	13	8	2	3	0.46
>65 y	19	7	4	8	
Sex					
Male	17	8	3	6	1.00
Female	15	7	3	5	
ECOG performance status					
0	24	11	4	9	0.87
1	8	4	2	2	
Metastatic stage					
M0	5	2	0	3	0.69
M1a	9	5	2	2	
M1b	3	2	1	0	
M1c	15	6	3	6	
Lactate dehydrogenase					
≤ULN	20	9	3	8	0.71
>ULN	12	6	3	3	
≤2xULN	28	13	5	10	1.00
>2xULN	4	2	1	1	
History of brain metastasis					
Yes	2	2	0	0	0.73
No	29	12	6	11	
Unknown	1	1	0	0	
Subtype					
Acral	15	7	2	6	0.81
Mucosal	17	8	4	5	
BRAF mutational status					
Mutation	4	3	0	1	0.64
No mutation	28	12	6	10	
Prior systemic therapy					
BRAF inhibitor	1	1	0	0	0.20
Chemotherapy	6	2	3	1	
None	25	12	3	10	
PD-L1 expression					
<5%	22	14	4	4	**0.004**
≥5%	10	1	2	7	

Abbreviations: ECOG, Eastern Cooperative Oncology Group; IDO, indoleamine 2,3-dioxygenase; PD-L1, programmed death ligand-1; ULN, upper limit of the normal range.
Bold values denote statistical significance at the $P < 0.05$ level.

up. A two-sided Fisher's exact test was used for exploring the association between clinical variables and the IDO expression and the association between the IDO expression and the best overall response. Cox-proportional hazard regression models were used to examine the relationships among IDO expression, PD-L1 expression and PFS with adjustment for possible confounders (the level of LDH and ECOG PS). Spearman's rank correlation was used to examine the relation between PD-L1 and IDO expression in tumors. $P < 0.05$ was considered statistically significant. Statistical analysis was performed using R statistical software (R Foundation for Statistical Computing).

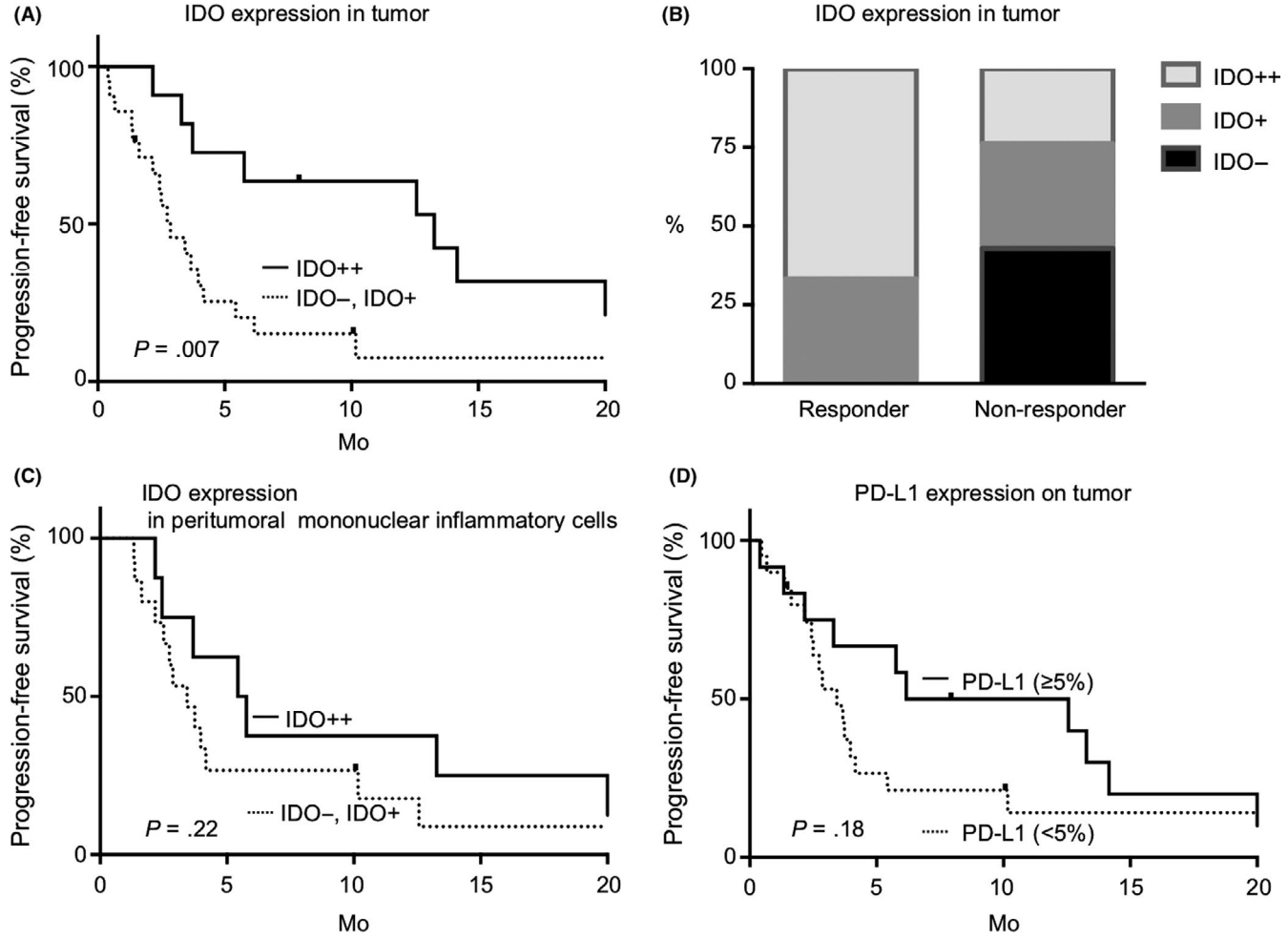

FIGURE 2 Association of indoleamine 2,3-dioxygenase (IDO) expression and programmed death ligand-1 (PD-L1) expression with response to anti–PD-1 therapy. The Kaplan-Meier progression-free survival (PFS) curve stratified by the IDO expression in tumor is shown (n = 32) (A). The IDO expression in tumors in responders and non–responders is shown (B). The Kaplan-Meier PFS curves stratified by the IDO expression in peritumoral mononuclear inflammatory cells (n = 23) (C) and PD-L1 expression on tumors (n = 32) (D) are shown. The log-rank test was performed for the univariate analysis

3 | RESULTS

3.1 | Patient characteristics

We enrolled 15 patients with acral melanoma and 17 patients with mucosal melanoma (Table 1). The median follow up was 10.3 months, with the database lock on 28 December 2017. This study included 17 men (53%), and the *BRAF*^{V600E} mutation was found in 4 patients (12.5%). A total of 15 patients (47%) had stage M1c disease, 12 patients (37.5%) had an elevated LDH level and 2 patients (6.2%) had brain metastases. A total of 7 patients (21.9%) had prior systemic treatments; 1 patient (3.1%) received BRAF inhibitor and 6 patients (18.8%) received dacarbazine-based chemotherapy. The median PFS was 3.73 months (3.96 months in acral melanoma and 3.73 months in mucosal melanoma). The ORR was 33% (95% confidence interval [CI]: 12%-61%) in acral melanomas and 26% (7.8%-55%) in mucosal melanomas.

3.2 | Indoleamine 2,3-dioxygenase and anti–programmed death ligang-1 were expressed by melanoma

Formalin-fixed, paraffin-embedded tumor specimens within 2 months before the first treatment of anti–PD-1 antibody were stained for IDO and PD-L1. IDO was expressed in tumors and in mainly inflammatory mononuclear cells of peritumoral tissues as determined by their morphologies. A total of 10 patients (31.2%) were classified as IDO–, 11 patients (34.4%) as IDO+ and 11 patients (34.4%) as IDO++ in tumors (Table 1) (Figure 1A-C). A total of 23 samples contained peritumoral tissues sufficient for evaluation of IDO expression in peritumoral tissue; 11 patients (47.8%) were classified as IDO–, 4 patients (17.4%) as IDO+ and 8 patients (34.8%) as IDO++ in peritumoral mononuclear inflammatory cells (Figure 1A-C). A total of 20 patients (87.0%) were classified as IDO positive and 3 patients (13.0%) as IDO negative in endothelial cells.

TABLE 2 Univariate Cox regression analysis of prognostic factors for progression-free survival in acral and mucosal melanoma patients

		HR	95%	*P*-value
Age	≤65 y vs >65 y	0.77	0.34-1.73	0.53
Sex	Male vs female	0.88	0.41-1.9	0.76
LDH	≤ULN vs >ULN	3.27	1.46-7.63	**0.004**
	≤2ULN vs >2ULN	5.03	1.59-15.94	**0.006**
ECOG performance status	0 vs 1	2.37	1.01-5.6	**0.04**
M stage	M0 vs M1a,b,c	2.64	0.78-8.9	0.11
History of brain metastasis	Absent vs present	4.57	0.47-44	0.18
Subtype	Acral vs mucosal	1.16	0.5-2.5	0.68
BRAF^{V600E} mutation	Absent vs present	0.58	0.17-1.96	0.38
Chemotherapy before nivolumab	Absent vs present	0.85	0.41-1.72	0.65
IDO expression in tumor	0, + vs ++	0.38	0.16-0.89	**0.026**
IDO expression in peritumoral inflammatory mononuclear cells	0, + vs ++	0.84	0.57-1.23	0.37
PDL1 expression on tumor	<5% vs ≥5%	0.60	0.27-1.33	0.21

Abbreviations: ECOG, Eastern Cooperative Oncology Group; IDO, indoleamine 2,3-dioxygenase; LDH, lactate dehydrogenase; PD-L1, programmed death ligand-1.
Bold values denote statistical significance at the $P < 0.05$ level.

TABLE 3 Multivariate Cox regression analysis of prognostic factors including IDO expression in tumor for progression-free survival in acral and mucosal melanoma patients

		HR	95% CI	*P*-value
LDH	≤ULN vs >ULN	3.97	1.65-9.54	**0.002**
ECOG performance status	0 vs 1	2.00	0.82-4.86	0.12
IDO expression in tumor	0, + vs ++	0.33	0.13-0.81	**0.016**

Abbreviations: ECOG, Eastern Cooperative Oncology Group; IDO, indoleamine 2,3-dioxygenase; LDH, lactate dehydrogenase
Bold values denote statistical significance at the $P < 0.05$ level.

A total of 12 cases (37.5%) were PD-L1-positive (Figure 1D,E). PD-L1 expression was positively correlated with IDO expression in tumors (rho = 0.53; $P < 0.01$, Spearman's rank correlation) (Table 1).

3.3 | Low expression of indoleamine 2,3-dioxygenase and in tumors was associated with poor progression-free survival and poor response to anti–programmed death-1 therapy

We examined whether the IDO and PD-L1 expressions were associated with response to anti–PD-1 antibody nivolumab in 32 acral and mucosal melanoma patients. We found that the low IDO expression in tumors was associated with poor PFS ($P = 0.007$, log rank test) (Figure 2A). The low expression of IDO in tumors showed a tendency toward poor PFS in acral melanomas (n = 15, $P = 0.027$) and mucosal melanomas (n = 17, $P = 0.113$). The median PFS was 13.3 months in the high IDO expression group and 2.9 months in the low IDO expression group. The IDO expression in tumors was independent of age, sex, ECOG PS, metastatic stage, LDH, a history of brain metastasis, subtype (acral or mucosal), *BRAF* mutational status and prior systemic therapy (Table 1). Univariate Cox-proportional hazard regression analysis revealed that an elevated LDH, ECOG PS and low expression of IDO were associated with poor PFS (Table 2). Multivariate Cox-proportional hazard regression analysis revealed that the low expression of IDO in tumors was significantly associated with poor PFS, after adjustments with the level of LDH and ECOG PS (hazard ratio [HR] = 0.33, $P = 0.016$, 95% CI = 0.13-0.81) (Table 3). Lower expression of IDO in tumors was observed in non–responders to anti–PD-1 therapy compared to responders ($P = 0.031$, by Fisher's exact test) (Figure 2B). The ORR was 54% (95% CI: 23%-83%) in the high IDO expression group and 15% (95% CI: 3.4%-39%) in the low IDO expression group. Although not statistically significant, cases with endothelial IDO expression in the peritumoral stroma showed a tendency toward longer PFS (Figure S1) (Table 4). We found that IDO expression in peritumoral mononuclear inflammatory cells was not associated with PFS (Figure 2C). Moreover, PD-L1 expression on tumor cells was not associated with PFS (Figure 2D) (Table 5). These results suggest that the IDO expression in tumors was correlated with response to anti–PD-1 therapy in acral and mucosal melanomas.

TABLE 4 Multivariate Cox regression analysis of prognostic factors including endothelial IDO expression in the peritumoral stroma for progression-free survival in acral and mucosal melanoma patients

		HR	95% CI	*P*-value
LDH	≤ULN vs >ULN	3.16	1.16-8.58	**0.02**
ECOG performance status	0 vs 1	2.16	0.75-6.20	0.15
Endothelial IDO expression	Negative vs positive	0.34	0.08-1.35	0.12

Abbreviations: ECOG, Eastern Cooperative Oncology Group; IDO, indoleamine 2,3-dioxygenase; LDH, lactate dehydrogenase.
Bold values denote statistical significance at the $P < 0.05$ level.

4 | DISCUSSION

In this study, we showed that the low expression of IDO in tumors was associated with poor PFS in Japanese acral and mucosal melanoma patients treated with anti–PD-1 therapy, whereas the PD-L1 expression on tumors was not associated with PFS. This finding suggests that assessment of the IDO expression could be useful for the identification of candidates for anti–PD-1 therapy among acral and mucosal melanoma patients, which comprise a great proportion of all melanomas in Asia.[15-19]

Indoleamine 2,3-dioxygenase facilitates immune escape by depleting tryptophan in tumor microenvironment.[25] There are conflicting reports regarding the relevance of IDO as a biomarker. A recent study demonstrated that the IDO expression in cutaneous melanoma was correlated with shorter PFS.[13] Another study found that endothelial expression of IDO was correlated with shorter overall survival.[23] On the other hand, Hamid et al[14] reported that IDO expression was associated with clinical response in advanced melanoma patients treated with anti–CTLA-4 antibody. Our finding is in accordance with the latter study; we found that the IDO expression in tumors was correlated with longer PFS in acral and mucosal melanoma patients treated with anti–PD-1 antibody. Moreover, cases with endothelial IDO expression showed a tendency toward longer PFS. This discrepancy in the role of IDO can be explained by the context of the studies. Studies reporting on the negative impact of IDO expression analyzed all melanoma patients treated surgically while we and Hamid et al studied melanoma patients undergoing specific cancer immunotherapy. Patients with IDO expression are likely to recur due to the immunosuppressive environment if surgically treated. If the patients are to be treated with cancer immunotherapy, IDO expression indicates that there is an immunosuppressive environment that can be reverted by immunotherapy. The IDO expression could be a candidate for biomarkers for response to anti–PD-1 therapy among acral and mucosal melanoma patients.

Interestingly, our data showed that the frequency of the IDO expression in tumors was lower in acral and mucosal melanomas than the reported frequency in cutaneous melanomas.[13] The genetic differences between acral and mucosal melanomas and cutaneous melanomas may contribute to the IDO expression levels in tumors and in the peritumoral stroma. Although anti–PD-1 inhibitor/IDO-1 inhibitor combination therapy in phase I/II trials had encouraging antitumor activity in metastatic melanomas,[26] the results of the phase 3 study did not detect the efficacy of them.[27] Because our results showed the varying IDO expression profiles between major melanoma subtypes, anti–PD-1 inhibitor/IDO-1 inhibitor combination therapy targeted for specific strata of melanoma patients may merit consideration.

Programmed death ligand-1 expression contributes to the impairment of effector functions of CD8+ tumor-infiltrating lymphocytes when it binds to PD-1.[28] Although PD-L1 is a well-studied biomarker for checkpoint inhibitors, some reports reveal that the PD-L1 status alone is not useful for the selection of patients for anti–PD-1 therapy.[2,3] We could not find an association between the PD-L1 expression on tumors and the prognosis in acral and mucosal melanoma patients treated with anti–PD-1 antibody. Therefore, the routine assessment of the IDO expression can be considered, particularly in acral and mucosal melanoma patients. There are several limitations in our study. First, a standardized method for the assessment of IDO expression is lacking, and this may adversely impact the generalizability of our study. Second, our study is limited by a small sample size (n = 32). Therefore, we propose that the relevance of biomarkers of the IDO expression in each subtype of melanoma should be evaluated in a large-scale study.

In summary, this study demonstrated that the low expression of IDO in acral and mucosal melanomas was associated with poor PFS in Japanese acral and mucosal melanoma patients treated with anti–PD-1 antibody. IDO could be a candidate for biomarkers for the response to anti–PD-1 therapy in acral and mucosal melanoma patients. Further study is needed to assess the clinical utility of our findings.

TABLE 5 Multivariate Cox regression analysis of prognostic factors including PD-L1 expression on tumor for progression-free survival in acral and mucosal melanoma patients

		HR	95% CI	*P*-value
LDH	≤ULN vs >ULN	3.13	1.24-7.87	**0.015**
ECOG performance status	0 vs 1	2.34	0.97-5.60	0.057
PDL1 expression on tumor	<5% vs ≥5%	0.90	0.37-2.21	0.83

Abbreviations: ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; PD-L1, programmed death ligand-1.
Bold values denote statistical significance at the $P < 0.05$ level.

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DISCLOSURE

The authors have no conflict of interest to declare.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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